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Abstract

With the electron microscopic and the negative staining method, the glomerular basement membrane of human and bovine kidneys was shown to have a definite fine meshwork structure. The pores of the meshwork of bovine glomerular basement membrane appeared to be pentagonal or hexagonal in shape. Strands of the meshwork branched three-dimensionally and made up the whole basement membrane. The portion of the sgrand between two neighboring branches was presumed to be a structural unit of the basement membrane. Glomerular basement membrane in man showed a structure similar to that seen in cattle, although the pore size of the meshwork was smaller in man than in cattle.

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—— BRIEF NOTE ——

ELECTRON MICROSCOPIC DEMONSTRATION OF MESHWORK STRUCTURE IN HUMAN AND BOVINE GLOMERULAR BASEMENT MEMBRANES

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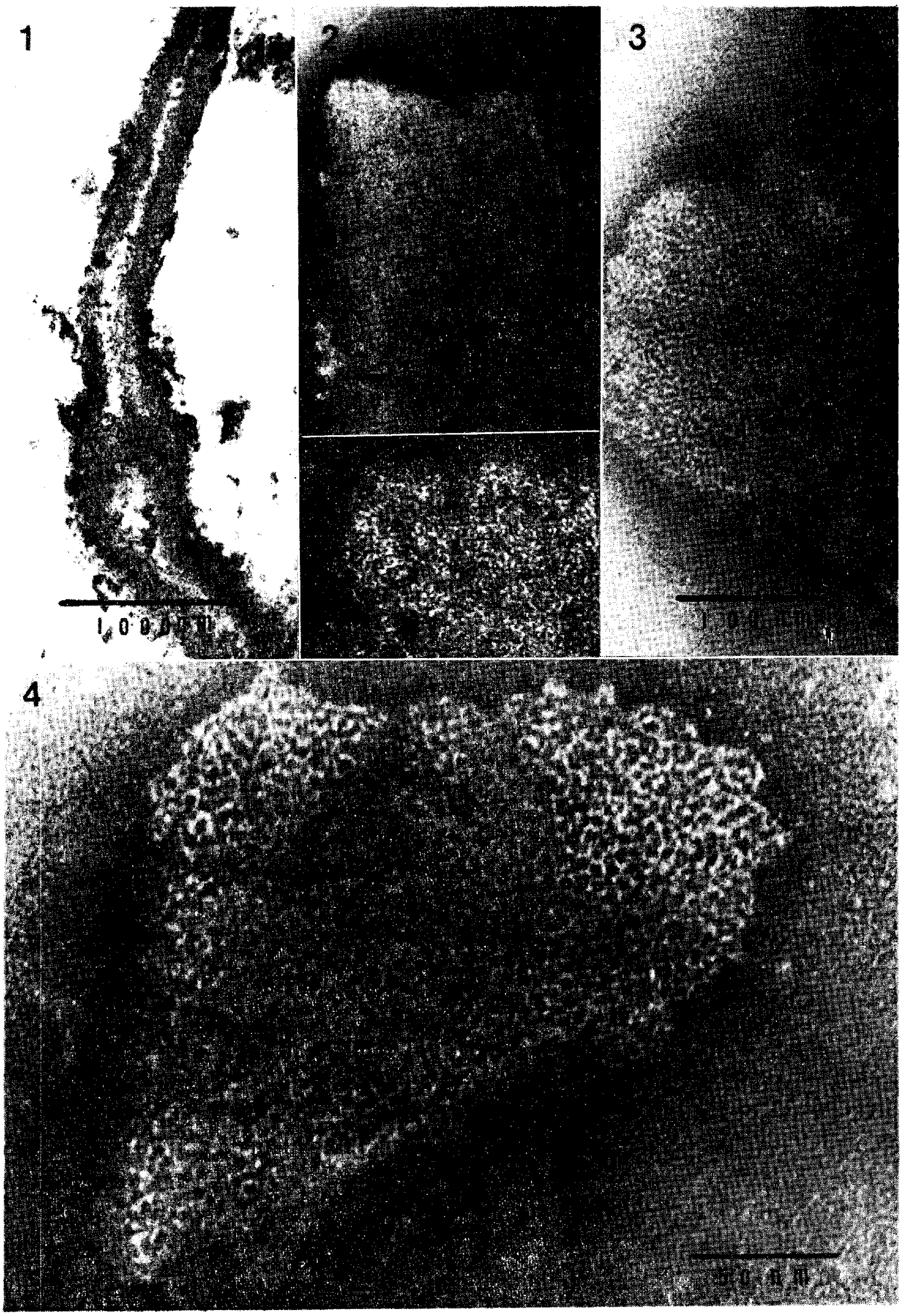
Abstract. With the electron microscopic and the negative staining method, the glomerular basement membrane of human and bovine kidneys was shown to have a definite fine meshwork structure. The pores of the meshwork of bovine glomerular basement membrane appeared to be pentagonal or hexagonal in shape. Strands of the meshwork branched three-dimensionally and made up the whole basement membrane. The portion of the strand between two neighboring branches was presumed to be a structural unit of the basement membrane. Glomerular basement membrane in man showed a structure similar to that seen in cattle, although the pore size of the meshwork was smaller in man than in cattle.

The glomerular basement membrane of the kidney is thought to be the main filtration barrier retaining plasma protein, and changes in its permeability appear to result in proteinuria (1). However, the morphological structure of the glomerular basement membrane is not well understood. Transmission electron microscope observation of the glomerular basement membrane indicated fine osmiophilic granules and filaments within the membrane. Electron microscopic observation by the freez-etching method showed that the glomerular basement membrane was composed of a regular and compact arrangement of fine granules, approximately 4 nm in diameter (2). Nevertheless, the ultrastructure of the glomerular basement membrane still needs to be clarified, and the membrane pores or channels by which low-molecular substances are filtrated from the capillary lumen to the urinary space of the Bowman's capsule have not been demonstrated. It is the purpose of this paper to report the ultrastructure of human and bovine glomerular basement membranes as seen with the negative staining method.


1
3
4
100 nm
100 nm
50 nm

Fresh bovine and human kidneys which had been obtained from autopsy cases without obvious renal diseases were stored at $-10°C$ until use. Isolation and purification of the glomerular basement membrane were performed according to the method of Krakower and Greenspon (3). The pellet containing glomerular basement membrane was first fixed by glutaraldehyde and osmium tetroxide and observed with a transmission electron microscope to confirm both that membrane was present and that contamination was minimal (Fig. 1). Then the pellet was submitted to electron microscopic observation by the negative staining method with 1.0% phosphotungstic acid, pH 7.3.

The results are shown in Figs. 2, 3 and 4. The upper micrograph in Fig. 2 shows the morphologic characteristics of renal basement membrane *i.e.*, a felt-like surface and linear contour with angular ends. Under higher magnification, this large fragment was composed of numerous small pores and strands (lower portion of Fig. 2). A regular meshwork struture can be clearly seen in a small fragment of bovine glomerular basement membrane (Figs. 3 and 4). The pores of the meshwork appear to be pentagonal or hexagonal in shape (Fig. 4) and measure approximately 4 nm in the shortest and approximately 4 to 8 nm in the longest diameters. The strands composing the meshwork measure approximately 1.6 nm in width. The meshwork is presumed to be "woven" by three-dimensional branchings of the strands. Therefore, the portion of the strands between two neighboring branches, 3 nm in length, is considered to be a structural unit of the meshwork. Human glomerular basement membrane has a structure similar to bovine membrane, but the diameter of the pores of the meshwork is somewhat smaller and measures approximately 3 nm.

To the best of our knowledge, the ulrastructural detail of the renal glomerular basement membrane has not been investigated by the negative staining method. In 1973, Olsen, Alper and Kefalides showed that a soluble fraction isolated from the basement membrane of the anterior lens capsule of sheep or bovine eyes was composed of strands and rounded particles by the negative staining (4). The strands demonstrated in our study are similar to the strands de-

Fig. 1. Pellet containing bovine glomerular basement membranes. Note the characteristic appearance of the membrane. Glutaraldehyde and osmium tetroxide fixation. ×24,000.

Fig. 2. The upper micrograph shows a portion of a large fragment of bovine glomerular basement membrane. The lower micrograph is an enlarged picture of a portion of the upper micrograph (arrow), showing numerous small pores and strands, 1.0% phosphotungstic acid negative staining. ×40,000 and ×200,000, respectively.

Fig. 3. Meshwork appearance in a small fragment of bovine glomerular basement membrane, 1.0% phosphotungstic acid negative staining. ×200,000.

Fig. 4. Meshwork structure in a small fragment of bovine glomerular basement membrane. The pores of the meshwork appear to be pentagonal or hexagonal in shape. ×400,000.

scribed in their report. In 1972, Misra and Berman proposed a molecular model of the glomerular basement membrane and suggested that the pores or channels of the basement membrane existed in the three dimensions as tortuous paths of the molecular model (5). Our results, however, demonstrate that the pores are far simpler than their theoretical proposal.

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